DTIC FILE COPY

(2)

TECHNICAL REPORT EL-89-16

US Army Corps of Engineers

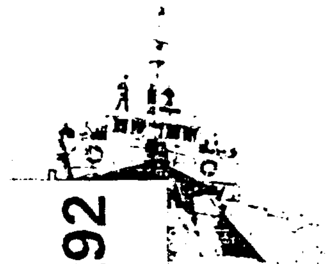
ENHANCEMENT OF PLANT ESTABLISHMENT ON DREDGED MATERIAL SITES WITH MYCORRHIZAL FUNGI AND CLAY AMENDMENTS

by

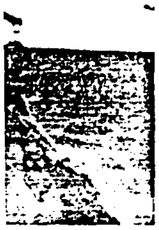
James K. Mitchell

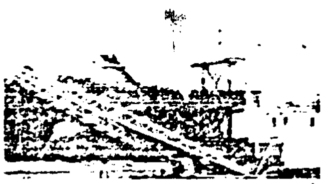
Environmental Laboratory

DEPARTMENT OF THE ARMY
Waterways Experiment Station, Corps of Engineers
3909 Halls Ferry Road, Vicksburg, Mississippi 39180-6199



AD-A214 292





US ARMY ENGINEER WATERWAYS EXPERIMENT STATION

October 1989

Final Report

ENVIRONMENTAL LABORATORY

Prepared for US Army Engineer District, Rock Island
Rock Island, Illinois 61204-2004

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Technical Report EL-89-16	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION USAEWES Environmental Laboratory; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) 3909 Halls Ferry Road Vicksburg, MS 39180-6199	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION USAED, Rock Island; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code) Rock Island, IL 61204-2004	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. / PROJECT NO. / TASK NO. / WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification)
Enhancement of Plant Establishment on Dredged Material Sites with Mycorrhizal Fungi and Clay Amendments

12. PERSONAL AUTHOR(S)
Mitchell, James K.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final report	FROM ______ TO ______	October 1989	33

16. SUPPLEMENTARY NOTATION
Available from National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Clay amendments; Plant establishment; (KT)
			Dredged material sites;
			Mycorrhizal fungi;

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The effect of vesicular-arbuscular mycorrhizae (VAM) and clay amendments on the growth in sandy substrates of selected plant species was investigated in greenhouse experiments and a field trial. VAM were applied as commercially available preparations of the fungi *Glomus deserticola*, *G. etunicatum*, and *G. intraradices*. The clays attapulgite, bentonite, kaolinite, and montmorillonite from various commercial sources were used as sediment amendments at rates from 5 to 15 percent (equivalent to 19 to 57 tons/acre).

Apparent host specificity, and the effects of inoculum density, fertilizer, clay type, clay particle size, and calcining of clays were evaluated in greenhouse experiments. In general, the 12 plant species tested (11 grasses and 1 herb) exhibited enhanced shoot and/or root growth when inoculated with any of the three VAM species. Only 4 of the 48 plant-VAM combinations did not result in higher plant yields than in uninoculated controls.

(Continued) over

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☑ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code) / 22c. OFFICE SYMBOL

DD Form 1473, JUN 86 Previous editions are obsolete. SECURITY CLASSIFICATION OF THIS PAGE

Unclassified

Keywords: [illegible]

19. ABSTRACT (Continued).

Maximum effect of VAM required an inoculum density of at least 25×10^3 chlamydospores/pot. Inoculation with VAM increased the yield of alfalfa at all fertilizer application rates tested (0.0 to 2.0 g Osmocote per liter of potting mix, equivalent to 0 to 2,500 lb/acre). Applied at rates of 5 to 15 percent (equivalent to 19 to 57 tons/acre) the clays bentonite and montmorillonite enhanced the growth of smooth brome grass. Top soil, the clays attapulgite and kaolinite, and the commercial products Agrosoke and Stawet were relatively ineffective in promoting plant growth. Montmorillonite of 16/30 and >40 mesh sizes enhanced growth of smooth brome grass more than coarser clay particles (6/30 mesh). Non-calcined montmorillonite promoted growth of smooth brome grass to a greater extent than calcined (heat-treated) montmorillonite.

Field trials evaluated the effect of VAM and clay amendments on establishment of seeded and transplanted plants in a sandy, upland dredged material disposal site near Oakville, IA. Trial plots received VAM inoculant, inoculant plus montmorillonite clay, or inoculant, clay, and oat straw mulch. Transplants in all treatments died, presumably owing to the severe drought which occurred during the summer of 1988. Plants became successfully established in the areas that received VAM/clay and VAM/clay/mulch treatment, but not in the VAM-only plot. At 160 days after planting, plant cover and biomass in the VAM/clay/mulch treated area (64 percent and 32 g/ft^2) were higher than in the VAM/clay area (42 percent and 18 g/ft^2).

The combination of VAM with clay amendments and mulch provides a practical, low-maintenance revegetation scheme for sandy dredged materials. It is recommended that sandy substrates be amended with ca. 25 tons montorillonite or bentonite clay per acre, that VAM inoculation be applied while seeding, and that mulching take place after planting. This regimen successfully revegetated sandy dredged materials without further attention during a severe drought and should readily succeed under more moderate conditions.

PREFACE

This study was sponsored by the US Army Engineer District, Rock Island (NCRDP). Mr. Jon Duyvejonck was the monitor for NCRDP.

The principal investigator during this study was Dr. James Mitchell, Aquatic Habitat Group (AHG), Environmental Resources Division (ERD), Environmental Laboratory (EL), US Army Engineer Waterways Experiment Station (WES). Assistance and support on this project were provided by Ms. Ramona Warren, Mr. Harvey Jones, Ms. Linda Winfield, and Drs. Gary Joye and Craig Smith, all of the AHG.

The work was conducted under the direct supervision of Dr. Alfred F. Cofrancesco, Jr., Leader of the Biomanagement Team and under the general supervision of Dr. John Harrison, Chief, EL, Conrad J. Kirby, Jr., Chief, ERD, and Mr. Edwin A. Theriot, Chief, AHG.

COL Larry B. Fulton, EN, was the Commander and Director of WES. Dr. Robert W. Whalin was Technical Director.

CONTENTS

CONVERSION FACTORS, NON-SI TO SI (METRIC)
UNITS OF MEASUREMENT

Non-SI units of measurement used in this report can be converted to SI (metric) units as follows:

Multiply	By	To Obtain
acres	4,046.873	square metres
cubic feet	0.02831685	cubic metres
feet	0.3048	metres
inches	25.4	millimetres
pounds (mass)	0.4535924	kilograms
square feet	0.09290304	square metres
tons (2,000 pounds, mass)	907.1847	kilograms

ENHANCEMENT OF PLANT ESTABLISHMENT ON DREDGED MATERIAL SITES WITH MYCORRHIZAL FUNGI AND CLAY AMENDMENTS

PART I: INTRODUCTION

Background

1. Revegetation of soils and dredged material consisting primarily of sand presents many problems (Adriani and Terwindt 1974; Biswas and Biswas 1977; Clarain et al. 1978; Malakouti, Lewis, and Stubbendieck 1978; Seneca, Woodhouse, and Brome 1976; Webster 1975; Whitfield and Brown 1948; Woodhouse, Seneca, and Brome 1977; and Woodhouse 1978). These materials are extremely low in organic matter, clay content, cation exchange capacity, and have very high internal water and air movement (Adriani and Terwindt 1974, Clar et al. 1981). Such adverse conditions promote nutrient depletion, warming, and rapid drying. In addition, these sites are subject to severe wind erosion. Amendments such as fertilizer and mulch are commonly used but provide only temporary relief and must often be repeated.

2. Success of sand-dune stabilizing (Koske, Sutton, and Sheppard 1975; Koske 1981; Koske and Halvorson 1981; Koske and Polson 1984; Nicolson and Johnston 1979; Sutton and Sheppard 1976) and semi-arid colonizing (Moorman and Reeves 1979, Trappe 1981) of plants often depends on the presence of compatible vesicular-arbuscular mycorrhizal fungi (VAM) in the substrate. Mycorrhizal fungi form a symbiotic association with plants and function similarly to root hairs, providing increased nutrient and moisture uptake to the plant (Schenk 1982). VAM also contribute to the aggregation of sand particles (Sutton and Sheppard 1976), thereby decreasing erosion potential.

3. Soil and clay amendments can also promote vegetation establishment by enhancing the water retention capacity and cation exchange capacity of sandy substrates. Used together, VAM and clay amendments have the potential to provide a low-maintenance revegetation scheme for sandy/upland dredge disposal sites. Both can be applied during the process of site preparation and planting to increase the establishment success of plants while decreasing the need to fertilize and irrigate.

Scope

4. This study examined the ability of combinations of VAM and clay amendments to promote growth of plant species in sandy, poorly watered substrates. Preliminary greenhouse studies evaluated various commercially available VAM inocula as well as clay and other amendments. The most promising combinations were evaluated in a pair of field demonstration plots, one using transplanted seedlings and one using plants that were seeded directly.

PART II: MATERIALS AND METHODS

Greenhouse Host Specificity Testing of VAM

5. All greenhouse tests were conducted in a nutrient-poor masonry sand (to simulate sandy/upland dredged material) having no recent history of use in agricultural production. The sand was analyzed by the Mississippi State Soil Testing Lab, Cooperative Extension Service, Mississippi State University. Results of the tests on the sand indicated a pH of 7.1 and 0.11 percent organic matter. Chemical elements present in the sand (pounds per acre*) were; P, 5; K, 9; Ca, 226; Mg, 44; S, 15; and Zn, 0.27. In addition, there was a 0.76 meq/g cation exchange capacity. The fertilizer Osmocote 17-9-13 (Sierra Chemical Company, Milpitas, CA) was incorporated at a rate of 0.25 g/ℓ sand.

6. Twelve plant species were selected by biologists at the US Army Engineer District, Rock Island (NCRDP) and scientists at the US Army Engineer Waterways Experiment Station (WES) as wildlife beneficial species for host specificity testing. Test plants included *Cynodon dactylon* (common Bermuda grass), *Bromus inermis* (smooth brome grass), *Sorghum sudanensis* (sudan grass), *Paspalum notatum* (bahia grass), *Festuca elatior* (tall fescue), *Dactylis glomerata* (orchard grass), *Schizachyrium scoparium* (little bluestem), *Eragrostis trichodes* (sand lovegrass), *Lolium perenne* (perennial rye), *Panicum virgatum* (switchgrass), *Agropyron smithii* (western wheatgrass), and *Portulaca oleracea* (common purslane).

7. Commercial formulations of the VAM species *Glomus etunicatum* (GE), *G. intraradices* (GI), and *G. deserticola* (GD) were obtained in a masonry sand carrier from Native Plants, Inc. (NPI), Salt Lake City, UT. Inocula of GE, GI, and GD contained 2,157, 1,116, and 1,080 chlamydospores/mℓ, respectively (inoculum densities were provided by NPI). Sufficient inoculum was added to each pot to deliver 1.5×10^4 chlamydospores/pot of each fungal species. Figure 1 illustrates the placement of VAM inoculum for host specificity testing. Test plants were grown on greenhouse benches at 25° to 32° C in 15- by 15-cm

---

* A table of factors for converting non-SI units of measurement to SI (metric) units is presented on page 3.

(6- by 6-inch) plastic pots (10 plants/pot). Each treatment was applied to four pots. Control plants were not inoculated with VAM.

8. The watering regime for experimental plants simulated drought stress such as might be expected on sandy/upland disposal sites. Plants were allowed to dry until symptoms of wilt appeared. When this occurred, plants were watered with reverse-osmosis water at a rate of 100 mℓ/pot. This watering regime was maintained until plant harvest. Plant height was measured at irregular intervals from emergence until the end of the experiment. After 75 days, plants were excised at the soil surface. Roots and leaves were oven-dried at 100° C for 24 hours and dry weights were determined.

9. Dry weight measurements and final plant heights were analyzed by Analysis of Variance (ANOVA) and means were compared using least-significant-difference analysis (Steel and Torrie 1980). The experiment was conducted twice.

VAM Titer Studies

10. GI chlamydospores (1,000 spores/mℓ) in a masonry sand carrier (from NPI) were applied at different densities to determine the inoculum levels required to enhance plant growth under conditions of nutrient stress. The inoculum concentration of GI was adjusted to deliver from 2×10^3 to 1×10^5 chlamydospores/pot. Test plants (orchard grass) were grown on greenhouse benches at 25° to 32° C in plastic pots (10 plants/pot) as described above. Each treatment was applied to four pots. Control plants were not inoculated with GI. Prior to planting and inoculation, the slow-release fertilizer Osmocote 17-9-13 was incorporated at a rate of 0.2 g/ℓ of sand. Plants were watered weekly at a rate of 150 mℓ/pot. After 75 days, plants were excised at the sand surface, oven-dried, and shoot dry weight was determined as described above. Dry weight measurements were analyzed by ANOVA and mean comparisons were made using least-significant-difference analysis (Steel and Torrie 1980). The experiment was conducted twice.

Effect of Fertilizer Concentration on VAM Efficacy

11. A range of application rates of the fertilizer Osmocote 17-9-13 was tested to determine the optimal fertilization rate for growth of VAM-colonized

and non-colonized plants. Fertilizer rates ranged from 0.25 to 2.0 g/ℓ of masonry sand. Fertilizer and sand were blended for 5 min in a portable cement mixer and placed into 15- by 15-cm plastic pots. Half of the pots were inoculated with GI at 1.5 x 10^4 chlamydospores/pot as described above. The remaining half of the pots were not inoculated. Test plants (alfalfa) were grown on greenhouse benches at 25° to 32° C (3 plants/pot). Each treatment consisted of 12 plants. Control plants were grown in sand without added fertilizer and were either inoculated or not inoculated with GI. Plants were watered weekly at a rate of 150 mℓ/pot. After 66 days plants were excised at the sand surface, oven dried, and shoot dry weights were determined as above. Dry weight measurements were analyzed by ANOVA and means were compared using least-significant-difference analysis (Steel and Torrie 1980). The experiment was conducted twice.

Interaction of VAM and Sediment Amendments

12. Several amendments that increase moisture retention and cation exchange capacity were tested to determine their effect on the growth of VAM-inoculated plants and those that were not inoculated. Tested amendments included the polyacrylamide gel Agrosoke (Grosoke International, Fort Worth, TX), the starch polymer Stawet (Polysorb, Mandeville, LA), top soil, and the clays bentonite, attapulgite, kaolinite, and montmorillonite. Trade name and company addresses for the clays are listed in Table 1. Agrosoke and Stawet were incorporated into masonry sand at label recommendations of 1 teaspoon/pot and 1 tablespoon/pot, respectively. Topsoil and clay amendment (>40 mesh) rates of incorporation and equivalences are listed in Table 2. Amendments and the fertilizer Osmocote 17-9-13 (0.2 g/ℓ sand) were blended with masonry sand for 5 min in a portable cement mixer and placed into plastic pots. Half of the pots were inoculated with GI at a rate of 1.5 × 10^4 chlamydospores/pot, while the remaining half were not inoculated. Test plants (smooth brome) were grown on greenhouse benches at 25° to 32° C (10 plants/pot). Each treatment consisted of four pots. Control plants were grown in sand with no added amendment and were either inoculated with GI or not inoculated. Plants were fertilized and maintained under simulated drought stress as described above. After 70 days, plants were excised at the sand surface, oven-dried, and shoot dry weights were determined as described above. Dry weight measurements were

analyzed by ANOVA and means were compared using Tukey's test (Steel and Torrie 1980). The experiment was conducted twice.

Effect of Clay Particle Size on Plant Growth

13. Smooth brome grass plants were grown in sand amended with different sizes of Oran-RVM montmorillonite clay (Lowes, Inc., South Bend, IN) to determine whether the size of added clay particles influences plant growth. Four mesh sizes were tested: 6/30, 16/30, and >40 (fines). Clay was incorporated into masonry sand at a rate of 40 kg/m^3 and Osmocote 17-9-13 was added at a rate of 0.2 g/ℓ of masonry sand. The sand-clay-fertilizer mixture was blended in a portable cement mixer for 5 min, then distributed into plastic pots as described above. Test plants were grown on greenhouse benches at 25° to 32° C (10 plants/pot). Each treatment was applied to four pots. Control plants were grown in sand with no clay amendment. Plants were maintained under simulated drought stress conditions as described above. After 75 days plants were excised at the sand surface, oven-dried, and shoot dry weights were determined as described above. Dry weight measurements were analyzed by ANOVA and means were compared using least-significant-difference analysis (Steel and Torrie 1980). The experiment was conducted twice.

Effect of Calcined vs. Non-Calcined Clay on Plant Growth

14. Growth of smooth brome grass plants in sand amended with Florco-LVM (calcined) and Florco-RVM (non-calcined) montmorillonite clays (Floridin Co., Norcross, GA) was compared to determine whether calcined (heat-treated) clays differ from non-calcined clays in their ability to promote plant growth. Clays (16/30 mesh) were incorporated into masonry sand at a rate of 40 kg/m^3 and Osmocote 17-9-13 was added at a rate of 0.2 g/ℓ of sand. Test plants were grown on greenhouse benches at 25° to 32° C (10 plants/pot). Each treatment was applied to 40 pots. Control plants were grown in sand with no clay amendment. Plants were maintained under simulated drought stress by the watering regime described above. After 70 days, plants were excised at the sand surface, oven-dried, and shoot dry weight was determined as described above. Dry weight measurements were analyzed by ANOVA and means were compared using Tukey's test (Steel and Torrie 1980). The experiment was conducted twice.

1988 VAM Field Demonstration

15. A 2-acre sandy/upland dredge disposal site on the Mississippi River near Oakville, IA, was chosen by NCRDP biologists and WES scientists for 1988 field demonstration trials.

16. Site preparation. On 1 May 1988 the entire site was leveled with a D3-Caterpillar bulldozer equipped with power takeoff (PTO). Sediment samples were collected for chemical analysis. On 16 May 1988 a Big-A lime spreader (rear applicator) was used to apply a mixture of 20-5-10 fertilizer (250 lb/acre), gypsum (650 lb/acre), and Sulpomag (150 lb/acre). Fertilizer nitrogen was 50 percent urea-N and 50 percent KNO_3-N. The rate of fertilizer application was chosen based on chemical analysis of sediment material.

17. Transplanted demonstration plots. The fertilizer mixture in half (1 acre) of the site was incorporated using a cutaway rolling disk with 1/4-in.-thick disks spaced 6 in. apart. The disk was pulled with a D3-Caterpillar bulldozer. The site was divided into 6-m^2 (20-ft^2) small demonstration plots. Amendments tested included the montmorillonite clays (>40 mesh) Florco-RVM (25 tons/acre) and Oran-RVM (25 tons/acre), and oat straw mulch (4,000 lb/acre). Clay amendments were disked into the plots prior to planting, whereas the mulch was broadcast between rows after planting. Control plots received no clay or mulch.

18. Smooth brome grass, tall fescue, and orchard grass plants for transplantation were grown from seed in 72-cell (1-1/4 by 1-1/4 by 1-1/2 in.) plastic growth trays within the greenhouse. Each cell contained approximately 10 plants. Half of the trays were previously inoculated with the commercial VAM (GI) inoculum Nutrilink (3 ℓ inoculum/300 ℓ potting mix), while the remaining half were not inoculated. Final concentration of GI was approximately 1,500 chlamydospores/cell. The potting mix was prepared by combining one 4-ft^3 bale of Sunshine No. 2 peat moss, one 4-ft^3 bale of horticultural grade perlite (for a total of 300 ℓ), and 150-g Osmocote 17-9-13.

19. On 16 May 1988 each plot was planted with 12 rows of 20 plant bunches each. Rows were 1 m (3.2 ft) apart and plants within the rows were 30 cm (1 ft) apart. Every plot contained all three plant species, each planted in four randomly selected rows. Treatment plots (eg. Florco-RVM) contained grass species that were either inoculated or not inoculated. There were four plots/treatment, arranged in a random complete block design. After

22 weeks, all plants within the two center rows of each grass species were excised at the sand surface, oven-dried, and root and shoot dry weights were determined as described above. Plant height was also recorded.

20. Seeded demonstration plots. The remaining 1 acre was subdivided into three equal plots. One-third received no amendments. Oran-RVM (>40 mesh) montmorillonite clay (25 tons/acre) was disked into the remaining two-thirds of the site. One of the clay-amended thirds also received oat straw mulch (4,000 lb/acre, uncrimped), which was broadcast after planting. The entire acre was planted with a mixture of 25 wildflower (15 lb/acre), 12 grass (21 lb/acre), and one legume (6 lb/acre) plus *Rhizobium* inoculant (Table 3). Seeds were drill-seeded at a rate of 42 lb/acre with a John Deere doublehopper grain drill containing seed in the back hopper and Nutrilink (GI) inoculum (30ℓ/acre) in the front hopper. The grain drill was adjusted to deliver GI at a 6-in. depth and seed at a 1- to 2-in. depth. Row width was 8 in. Figure 2 is a photograph of the site just after planting.

21. Percent ground coverage and biomass (dry weight) were measured 160 days after planting. Each treatment area (1/3 acre) was divided into a grid of 1.0 m^2 squares and 16 squares in each treatment area were randomly selected. Percent ground cover within each selected grid square was estimated visually and all plants were collected from 1.0 ft^2 in the center of the square. Roots and shoots were separated, and dry weights were determined as described above. Cover and biomass measurements were analyzed by ANOVA and means were compared using least-significant-difference analysis (Steel and Torrie 1980).

PART III: RESULTS

Host Specificity Studies

22. Shoot dry weight of VAM-inoculated plants was nearly always significantly greater than that of control plants that were not inoculated (Figure 3). Only one species, western wheatgrass, did not exhibit a significant shoot response to inoculation with at least one of the VAM formulations. In general, shoot growth responded similarly to all three VAM formulations (GE, GD, and GI). Important exceptions to this were little bluestem and common purslane, which did not exhibit a significant response to GI, and common Bermuda grass and perennial rye grass, which did not respond to GD and GI.

23. The response of root dry weight to inoculation with VAM was similar to that of shoots (Figure 4). Except for two species, sudan grass and tall fescue, root production of all plant species was stimulated by at least one of the three VAM species, compared with uninoculated controls. Only a few plant species responded differently to the three species of VAM. Orchard grass produced the most root biomass when inoculated with GE, common Bermuda grass with GI, and smooth brome grass with GD.

24. When the effects of VAM on root and shoot production are considered together, most VAM-plant combinations result in increased production of shoots, roots, or both, as compared with uninoculated control plants (Table 4).

25. Plant height measurements (data not shown) corroborated shoot dry weight measurements, but were less sensitive to differences among the responses of a single plant species to different species of VAM.

Titer Studies

26. In treatments having concentrations of 25×10^3 chlamydospores per pot and higher, shoot dry weight of orchard grass did not differ significantly among treatments (Figure 5). Concentrations below this level resulted in significantly reduced yields, e.g. the yield of control plants was only about one-fifth that of those receiving 25×10^3 chlamydospores/pot.

Fertilizer Studies

27. The yield of shoots and roots of VAM-inoculated plants was greater than that of uninoculated controls at virtually all rates of fertilization (Figure 6). Increasing fertilization prompted increased shoot and root yields up to approximately 1.0 g/ℓ. Above 1.0 g/ℓ yields were constant or decreased slightly in response to higher levels of fertilization.

Interaction of VAM and Sediment Amendments

28. Growth of smooth brome grass varied considerably in the various amendment treatments (Figure 7). Two-way ANOVA with amendment (type and rate) and VAM as factors indicated that (a) inoculation with VAM increased plant yield, (b) inoculation with VAM had approximately the same effect in all amendment treatments, and (c) the amendments differed in their effect on plant growth. In statistical terms, the analysis detected highly significant ($P<0.01$) effects of VAM and amendment, but the amendment x VAM interaction was not a significant source of variation. Plant yield was greatest in the 15 percent montmorillonite treatments and in the 10 and 15 percent bentonite treatments, all of which grew significantly more than the unamended controls.

29. Comparison of the effect of clay types (plus topsoil), rates and inoculum with VAM by three-way ANOVA revealed that: (a) plant growth was significantly influenced by clay type, rate of application, and inoculation with VAM, (b) plant growth was more responsive to rate of application of some clays than others, and (c) the effect of VAM differed among the clay types.

30. Taken as a whole, the results of these analyses demonstrate that clay amendments and inoculation with VAM promote plant growth. Application of both VAM and clay amendments generally produces an effect that is approximately the sum of the individual effects. The clays bentonite and montmorillonite were the most effective in enhancing plant growth. Of the two, montmorillonite was more sensitive to the rate of application and declined in effectiveness more rapidly at lower application rates than bentonite.

Clay Particle Size Studies

31. The growth response of smooth brome grass to varying sizes of Oran RVM montmorillonite clay is shown in Table 5. Masonry sand amended with 6/30 mesh montmorillonite clay at 40 kg/m^3 did not significantly increase shoot dry weight of smooth brome grass, compared to unamended controls. However, amending sand with either 16/30 or >40 mesh clays (at comparable rates) did significantly increase growth of smooth brome grass over controls.

Calcined vs. Non-Calcined Clay Studies

32. Growth of smooth brome grass under conditions of drought stress in calcined and non-calcined montmorillonite clay is presented in Table 6. Smooth brome grass grew significantly better in either calcined (Florco-LVM) or noncalcined (Florco-RVM) clay-amended sediment than within non-amended controls. However, shoot dry weight production was significantly greater in Florco-RVM than in Florco-LVM.

1988 VAM Field Studies

33. On 30 June 1988 (44 days after planting) the field demonstration site was surveyed. All transplanted seedlings within the small demonstration plot area had perished. The area was suffering from a severe drought, and the transplants had probably been killed by extreme water stress. Within the VAM/clay/mulch portion of the seeded demonstration plot, a few scattered grass and wildflower seedlings had emerged. These were severely drought stressed, as evidenced by wilting and burned or curled leaf tips.

34. The site was again visited on 14 October 1988 (Figures 8-12). By this time plants had emerged and were growing in the VAM/clay/mulch and VAM/clay amended areas of the seeded demonstration plot. Plants were absent from the area amended with VAM only. A greater number of plant species (goosegrass, sand dropseed, sand lovegrass, giant foxtail, crabgrass, and purple lovegrass) were found growing within the VAM/clay/mulch amendment area as compared to the VAM/clay amendment area (sand lovegrass and sand dropseed).

35. By 26 October 1988 (160 days after planting) plants were still absent from the VAM-only treatment, but the VAM/clay and VAM/clay/mulch areas

were partially vegetated and supported measurable biomass. The VAM/clay/mulch site supported significantly greater total dry weight and percent cover than either the VAM/clay or the VAM sites (Table 7).

PART IV: DISCUSSION

36. Test results demonstrate that (a) inoculation with VAM and amendment with various clays can increase the growth of plants on sandy substrates under simulated drought stress, and (b) sandy dredged material can be successfully vegetated using VAM, clay, and mulch treatments. The combination VAM/clay/mulch treatment requires little maintenance and succeeded even during the severe drought experienced during the summer of 1988.

37. The first step in revegetation of a dredge disposal site is the choice of plant species, which is dependent on site use. Once this is accomplished, a commercial formulation of VAM that is compatible with the selected plant species must be selected. Some compatible plant-VAM combinations are listed in Table 4. Most of the plant-VAM combinations tested enhanced the growth of roots and/or shoots. Thus, when specific information on compatibility is not available, any of the three commercial VAM formulations can be used with a reasonable likelihood of success.

38. A minimum chlamydospore concentration of 25×10^3 spores/pot was required to achieve the maximum benefit from inoculation with VAM in greenhouse studies. A lower titer could be used in field demonstrations because greenhouse trials lasted only 75 days, while VAM would have an entire growing season (e.g. 150 days) or longer in which to become established in the field. Application of suboptimal spore concentrations may lead to the establishment of VAM on plants, but plant response will be delayed. Since rapid establishment is probably critical to the successful vegetation of hostile environments such as dredged material, inoculum application rates should not be reduced below those used in this study without evidence demonstrating that lower rates can succeed.

39. Fertilization with 1.0 g/ℓ of Osmocote 17-9-13 (microcapsules) was optimal for the growth of VAM-inoculated alfalfa as well as alfalfa that was not inoculated. Inoculation with VAM produced an increase in both shoot and root growth at nearly all fertilization rates. Other plant species should be tested to determine whether other VAM-plant combinations respond similarly.

40. In greenhouse tests, both VAM inoculation and amendment with clay or soil increased plant yield. Application of montmorillonite or bentonite clay with VAM increased growth to a greater extent than either did alone. Kaolinite and attapulgite clays and the commercial products Stawet and

Agrosoke did not enhance the growth of smooth brome grass at rates tested and are not likely to promote vegetation of dredged materials. Ideally, rates of at least 10-15 percent (38-57 tons/acre) should be applied to provide moisture retention and cation exchange capacity. In the seeded demonstration plot non-calcined montmorillonite clay (>40 mesh) was applied at a rate of 25 tons/acre, which was equivalent to adding a 1/2-in. layer of clay to the sediment surface. This rate was sufficient to allow successful plant establishment, even during the severe drought of 1988.

41. Clay particle sizes 16/30 mesh and smaller (>40) promoted growth of smooth brome grass to a greater extent than larger particle sizes. Disking the very fine >40 mesh clay into the surface of the demonstration site created a dust hazard. It is recommended that future field applications use clay particles of 16/30 mesh size. Non-calcined clay enhanced plant growth more effectively than calcined clay.

42. Vegetation of sandy/upland dredge disposal sites with transplanted seedlings was not successful, even with a full complement of VAM, clay, and mulch. The central United States experienced a severe drought during the summer of 1988 and an effort was made to water plants with a 1-1/2-in. gas-powered water pump, but this did not provide sufficient moisture to enable plants to survive. In other types of dredge material, or under less stringent environmental conditions, transplantation might succeed. Even if it could succeed, the amount of labor required to raise and transplant seedlings makes this method of revegetation uneconomical.

43. Drill seeding successfully vegetated dredged material when combined with sediment amendments or with sediment amendments and mulch. Apparently seeds were able to survive in a dormant state until sufficient rainfall had occurred to allow plant establishment. Without clay amendment, inoculation with VAM did not result in successful establishment of plants from seed. VAM/clay addition allowed plants to become established but was not as successful in promoting revegetation as VAM/clay/mulch additions. Either combination, VAM/clay or VAM/clay/mulch, provides a low-maintenance, hence economical, revegetation scheme.

PART V: CONCLUSIONS AND RECOMMENDATIONS

44. For optimal revegetation success, sandy upland dredged material sites should be amended with 16/30 mesh non-calcined montmorillonite or bentonite clay at a rate of at least 25 tons/acre (equivalent to adding approximately 1/2 in. of clay) to provide moisture retention and cation exchange capacity.

45. Increased plant growth and VAM establishment can be anticipated if sites are fertilized before planting.

46. Seeding is a more economical and effective means of revegetating sandy upland disposal sites than transplantation, particularly at locations where it would be difficult or impossible to water transplants regularly.

47. VAM inoculum should be applied at the time of seeding at a rate of at least 30 ℓ/acre (7.5 gal/acre). When specific information on plant-fungus compatibility exists, a VAM species that is compatible with the desired plant species should be selected. When information on compatibility is unavailable, the results of this study suggest that commercially available VAM inoculum can be expected to work with most plant species.

48. Mulching with oat straw or a similar material after planting can increase the success of revegetation efforts.

49. A John Deere 777 air seeding system in combination with either a 610 or 1060 seeding tool would be a more efficient means of applying VAM and seeds than the John Deere double-hopper grain drill used in the 1988 field demonstration. As with the implements used in this study, a D3 Caterpillar bulldozer equipped with PTO is required.

REFERENCES

Adriani, M. J., and Terwindt, J. J. 1974. "Sand Stabilization and Dune Building," Rykswaterstaat Communication, No. 19, Government Publishing Office, Hague, Netherlands.

Biswas, M. R., and Biswas, A. K. (editors). 1977. "Desertification," Environmental Sciences and Applications, Vol 12, Pergamon Press, Oxford, 523 pp.

Clar, M. L., Das, P., Ferrandino, J., and Barfield, B. 1981. Handbook of Erosion and Sediment Control Measures for Coal Mines, US Department of the Interior, Office of Surface Mining Reclamation and Enforcement, Washington, DC.

Clarain, E. J., Cole, R. A., Diaz, R. J., Ford, A. W., Huffman, R. T., Hunt, L. J., and Wells, B. R. 1978. "Habitat Development Field Investigations, Miller Sands Marsh and Upland Habitat Development Site, Columbia River, OR," Technical Report D-77-38, US Army Engineer Waterways Experiment Station, Vicksburg, MS.

Koske, R. E., Sutton, J. C., and Sheppard, B. R. 1975. "Ecology of *Endogone* in Lake Huron Sand Dunes," Canadian Journal of Botany, Vol 53, pp 87-93.

Koske, R. E. 1981. "A Preliminary Study of Interactions Between Species of Vesicular-Arbuscular Fungi in a Sand Dune," Transactions of the British Mycological Society, Vol 76, pp 411-416.

Koske, R. E., and Halvorson, W. L. 1981. "Ecological Studies of Vesicular-Arbuscular Mycorrhizae in a Barrier Sand Dune," Canadian Journal of Botany, Vol 59, pp 1413-1422.

Koske, R. E., and Polson, W. R. 1984. "Are VA Mycorrhizae Required for Sand Dune Stabilization?," Bioscience, Vol 34, pp 420-424.

Malakouti, M. J., Lewis, D. T., and Stubbendieck, J. K. 1978. "Effect of Grasses and Soil Properties on Wind Erosion in Sand Blowouts," Journal of Range Management, Vol 31, pp 417-420.

Moorman, T., and Reeves, F. B. 1979. "The Role of Endomycorrhizae in Revegetation Practices in the Semi-Arid West, II. A Bioassay to Determine the Effect of Land Disturbance on Endomycorrhizal Populations," American Journal of Botany, Vol 66, pp 14-18.

Nicolson, T. H., and Johnston, C. 1979. "Mycorrhizae in the Gramineae, III. *Glomus fusiculatus* as the Endophyte of Pioneer Grasses in a Maritime Sand Dune," Transactions of the British Mycological Society, Vol 72, pp 261-268.

Schenck, N. C. 1982. Methods and Principles of Mycorrhizal Research, American Phytopathological Society, St. Paul, MN, 244 pp.

Seneca, E. D., Woodhouse, W. W., and Brome, S. W. 1976. "Dune Stabilization with *Panicum amarum* Along the North Carolina Coast," Miscellaneous Report No. 76-3, US Army Engineer Coastal Engineering Research Center, Fort Belvoir, VA.

Steel, R. G. D., and Torrie, J. H. 1980. Principles and Procedures of Statistics, 2nd ed., McGraw-Hill, New York, 633 pp.

Sutton, J. C., and Sheppard, B. R. 1976. "Aggregation of Sand-Dune Soil by Endomycorrhizal Fungi," Canadian Journal of Botany, Vol 54, pp 326-333.

Trappe, J. M. 1981. "Mycorrhizae and Productivity of Arid and Semi-Arid Rangelands," Advances in Food Producing Systems for Arid and Semi-Arid Lands, Academic Press, Inc., New York, pp 581-599.

Webster, A. N. 1975. "Prairie Grasses, the Vista-Makers," Weeds, Trees, and Turf, Vol 14, pp 34-35.

Whitfield, C. J., and Brown, R. L. 1948. "Grasses that Fix Sand Dunes," Yearbook of Agriculture, US Government Printing Office, Washington, DC.

Woodhouse, W. W., Seneca, E. D., and Brome, S. W. 1977. "Ten Years of Development of Man-Initiated Coastal Barrier Dunes in North Carolina," UNG-SG-77-01, University of North Carolina Sea Grant College, Raleigh, NC.

Woodhouse, W. W. 1978. "Dune Building and Stabilization with Vegetation," Special Report No. SR-3, US Army Engineer Coastal Engineering Research Center, Fort Belvoir, VA.

Table 1

Trade Name and Company Addresses for Clays Tested

Clay Type	Trade Name	Company
Attapulgite	Attagel 350	Engelhard, Menlo Park, CA
Attapulgite	Florkleen	Floridin Co., Quincy, FL
Kaolinite	Kaolin RC-32	Thiele Kaolin Co., Wrens, GA
Montmorillonite	Florco-RVM	Floridin Co., Quincy, FL
	Florco-LVM	
Montmorillonite	Oran-RVM	Lowe's, Inc., South Bend, IN
Bentonite	Creek-O-Nite	Lowe's, Inc., South Bend, IN

Table 2

Topsoil and Clay Amendment Rates of Incorporation

Percent Rate	$lb/1,000\ ft^2$	tons/acre	kg/m^3	kg/Ha
5	875	19	29.9	42,700
10	1,750	38	59.8	85,400
15	2,625	57	89.7	128,100

Table 3

Plant Species Used in Drill-Seeded Demonstration Plot

Scientific Name	Common Name	lb/acre
Bromus inermis	Smooth brome	5
Andropogon gerardi	Big bluestem	1
Schizachyrium scoparius	Little bluestem	3
Festuca arundinacea	Tall fescue	5
Dactylis glomerata	Orchard grass	1
Eleusine indica	Goosegrass	0.5
Cynodon dactylon	Common Bermuda	1
Sporobolus cryptandrus	Sand dropseed	0.5
Eragrostis trichodes	Sand lovegrass	2
Setaria faberii	Giant foxtail	1
Digitaria sanguinalis	Crabgrass	0.5
Eragrostis spectabilis	Purple lovegrass	0.5
Trifolium pratense	Red clover	6
Midwestern Wildflower Mix*		15
Achillea millefolium	Yarrow	
Aster laevis	Smooth aster	
Aster tanacetifolius	Prairie aster	
Asclepias tuberosa	Butterfly weed	
Coreopsis lanceolata	Coreopsis	
Coreopsis tinctoria	Plains coreopsis	
Echinacea pallida	Pale purple coneflower	
Echinacea purpurea	Purple coneflower	
Eryngium yuccifolium	Rattlesnake master	
Gaillardia pulchella	Firewheel	
Gypsophila elegans	Baby's breath	
Linum lewisii	Blue flax	
Linaria maroccana	Baby snapdragon	
Liatris pycnostachya	Thickspiked gayfeather	
Liatris spicata	Spiked gayfeather	
Lupinus perennis	Sundial lupine	
Lupinus texensis	Texas bluebonnet	
Monarda fistulosa	Wild bergamot	
Oenothera missouriensis	Missouri evening primrose	
Papaver rhoeas	Flanders poppy	
Petalostemon purpureum	Purple prairie clover	
Phlox drummondii	Drummond phlox	
Ratibida columnaris	Prairie coneflower	
Ratibida pinnata	Grayhead prairie coneflower	
Rudbeckia hirta	Black-eyed Susan	

* Seed mixture obtained from: Native Plants Incorporated, Salt Lake City, Utah.

Table 4

VAM-Plant Combinations Producing Significantly Enhanced Growth Compared With Uninoculated Controls

Plant Species	VAM Species GD	VAM Species GI	VAM Species GE
Tall fescue	S*	S	S
Orchard grass	S	B**	B
Little bluestem	B	R†	S
Sand lovegrass	S	B	B
Common Bermuda grass	S	B	S
Smooth brome grass	B	B	B
Sudan grass	S	S	S
Bahia grass	B	B	B
Perennial rye grass	R	-††	B
Switch grass	B	B	B
Western wheatgrass	R	-	-
Common purslane	S	-	S

* S = enhanced growth of shoots.
** B = enhanced growth of both shoots and roots.
† R = enhanced growth of roots.
†† - = enhanced growth of neither shoots nor roots.

Table 5

Growth Response of Smooth Brome Grass to Varying Sizes of Montmorillonite

Mesh Size	Shoot Dry Weight, g
6/30	1.22 ab*
16/30	1.74 a
>40	2.16 a
Control (no clay)	0.45 b

* Means followed by the same letter are not significantly different at P = 0.05.

Table 6

Growth of Smooth Brome Grass in Sand Amended with Calcined (LVM) and Non-Calcined (RVM) Clay Under Simulated Drought Stress

Treatment	Shoot Dry Weight, g
Florco-RVM	1.01 a*
Florco-LVM	0.55 b
Control (no clay)	0.05 c

* Means followed by the same letter are not significantly different in a Tukey test at P = 0.05.

Table 7

Plant Dry Weight and Percent Cover in the Seeded Demonstration Plots

Treatment	Total Dry Weight, g/ft^2	Percent Coverage
VAM/clay/mulch	31.7 a*	63.9 a
VAM/clay	18.1 b	41.6 b
VAM	0 c	0 c

* Within a column, means followed by the same letter are not significantly different at P = 0.05.

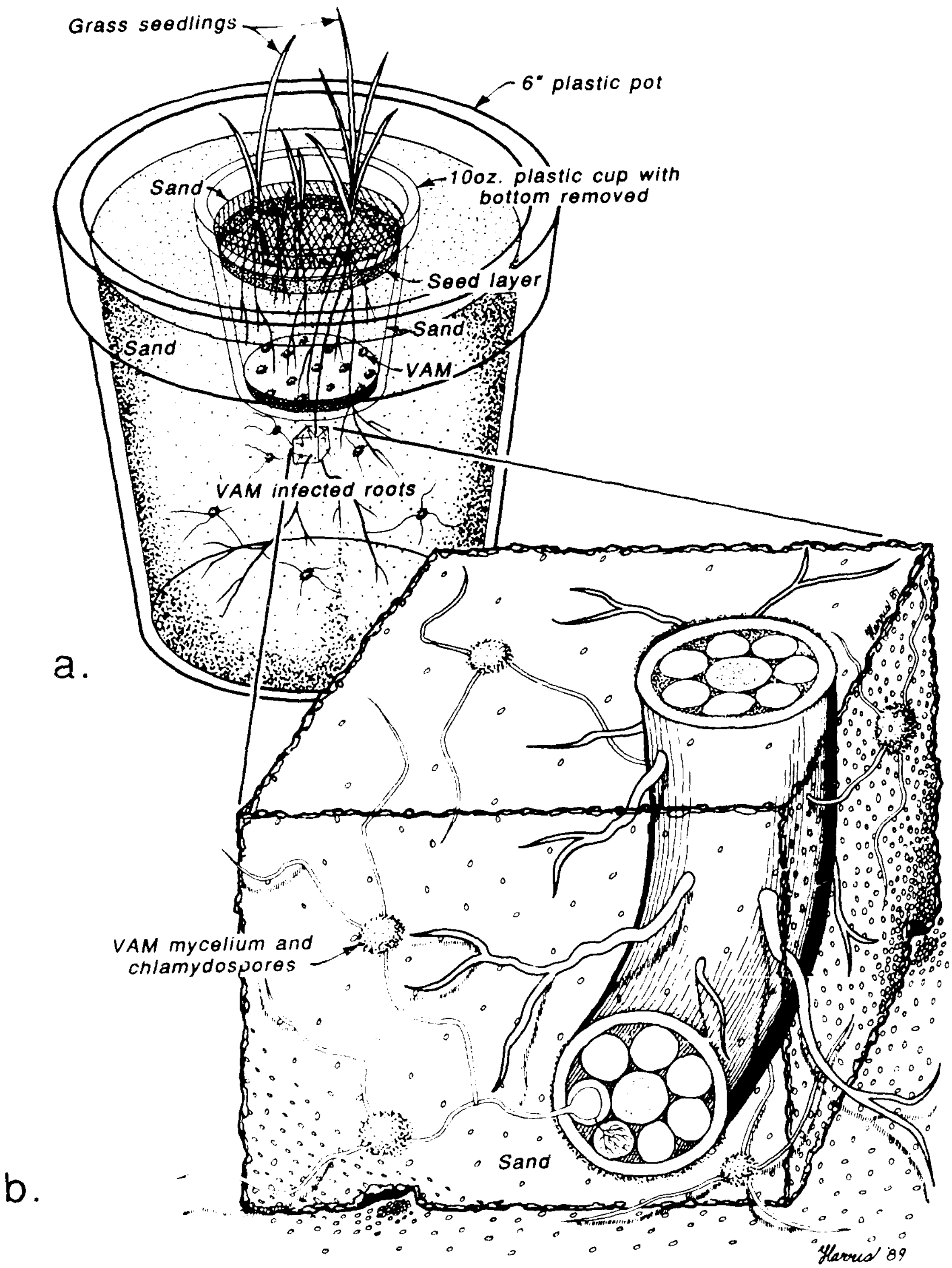


Figure 1. Illustration of (a) the potting system used to inoculate plant roots with vesicular-arbuscular mycorrhizae (VAM) for host range studies, and (b) the association between VAM and colonized roots

Figure 2. 1988 field demonstration plot. Small demonstration plots are on the left, large demonstration plots on the right. The VAM/clay/mulch area is in the right background

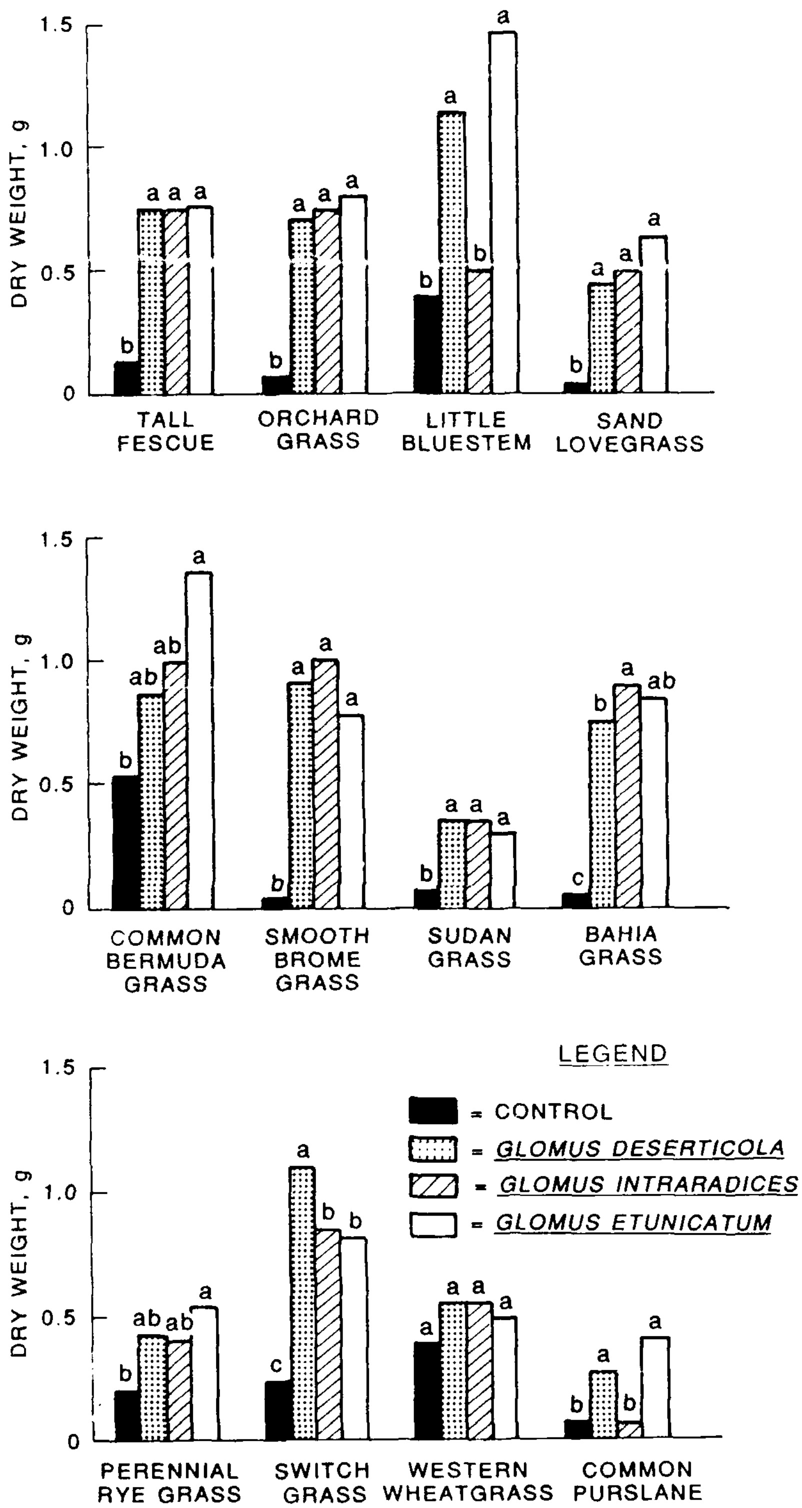


Figure 3. Aboveground (shoot) dry weight of the selected plant species inoculated with commercial VAM formulations. For each plant species, bars with the same letter are not significantly different at P = 0.05 in a least-significant-difference test

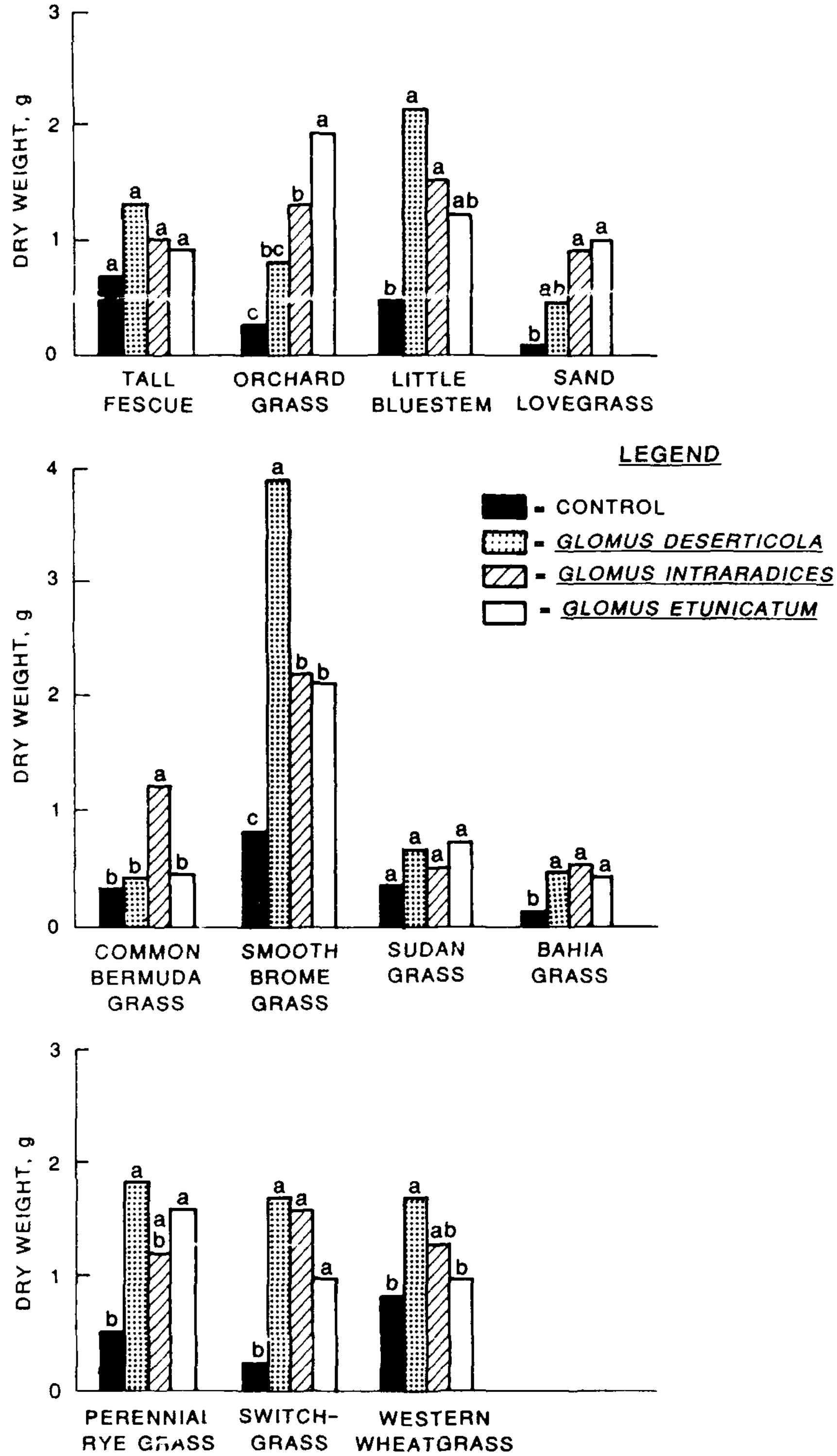


Figure 4. Below-ground (root) dry weight of the selected plant species inoculated with commercial VAM formulations. For each plant species, bars with the same letter are not significantly different at P = 0.05 in a least-significant-difference test

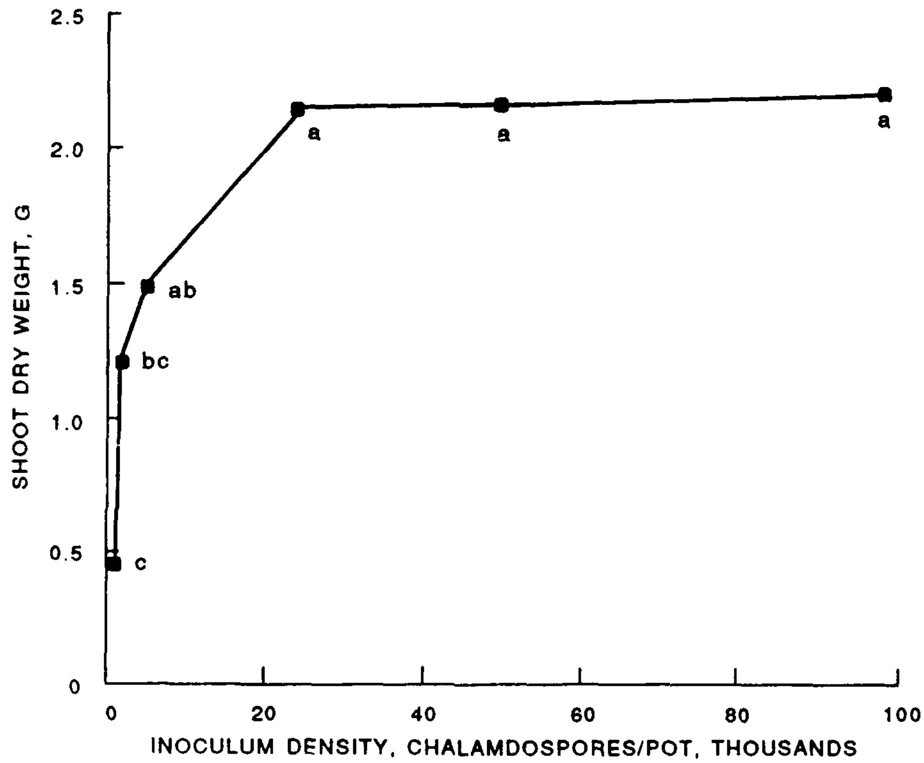


Figure 5. Effect of the density of *Glomus Intraradices* chlamydospores used as inoculum on the shoot yield of orchard grass. Points with the same letter are not significantly different at $P = 0.05$ in a least-significant-difference test

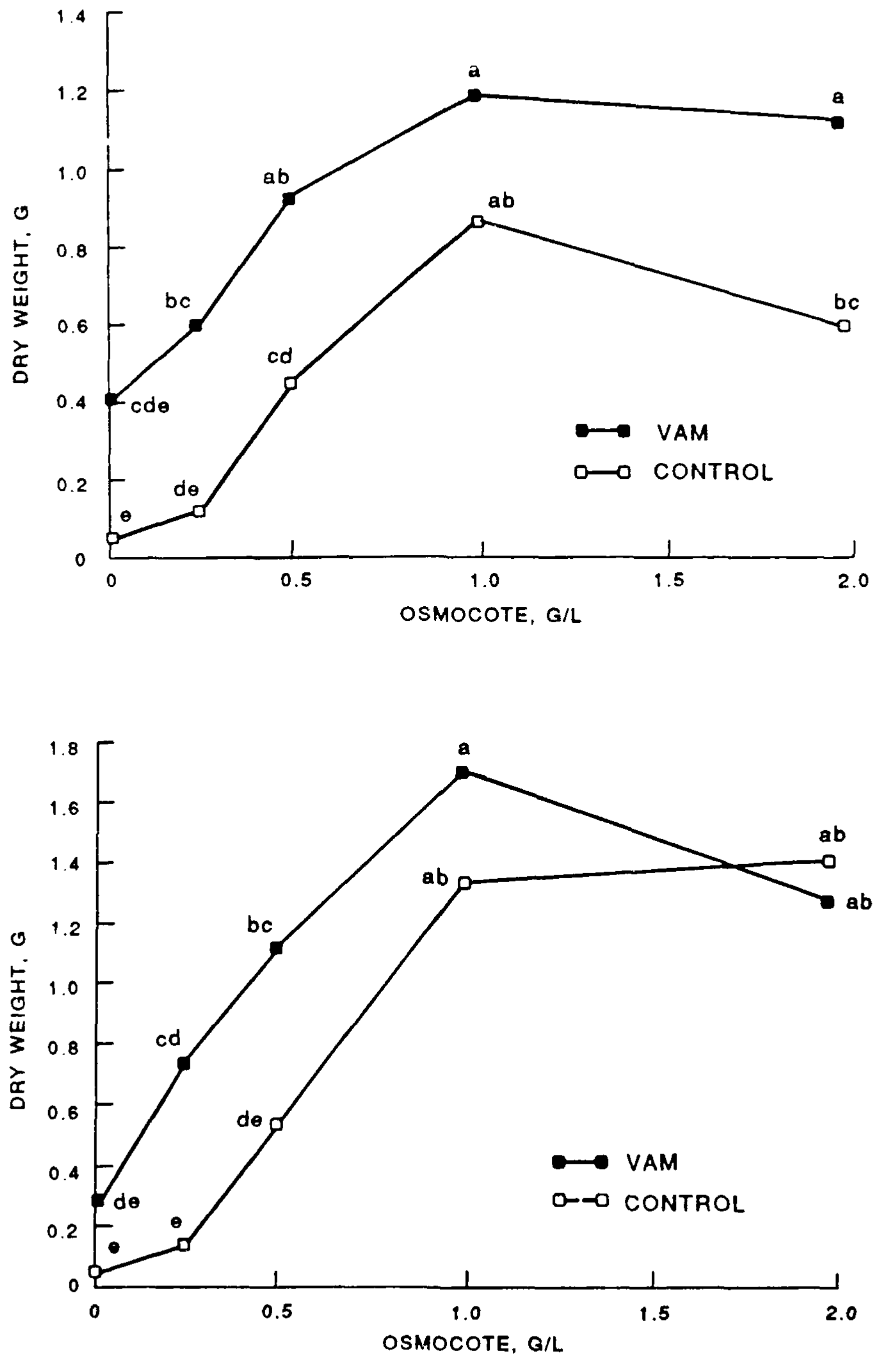


Figure 6. Effect of varying rates of fertilization on the yield of (a) shoots, and (b) roots of VAM-inoculated and uninoculated (control) alfalfa plants. Points with the same letter are not significantly different at P = 0.05 in a Tukey test

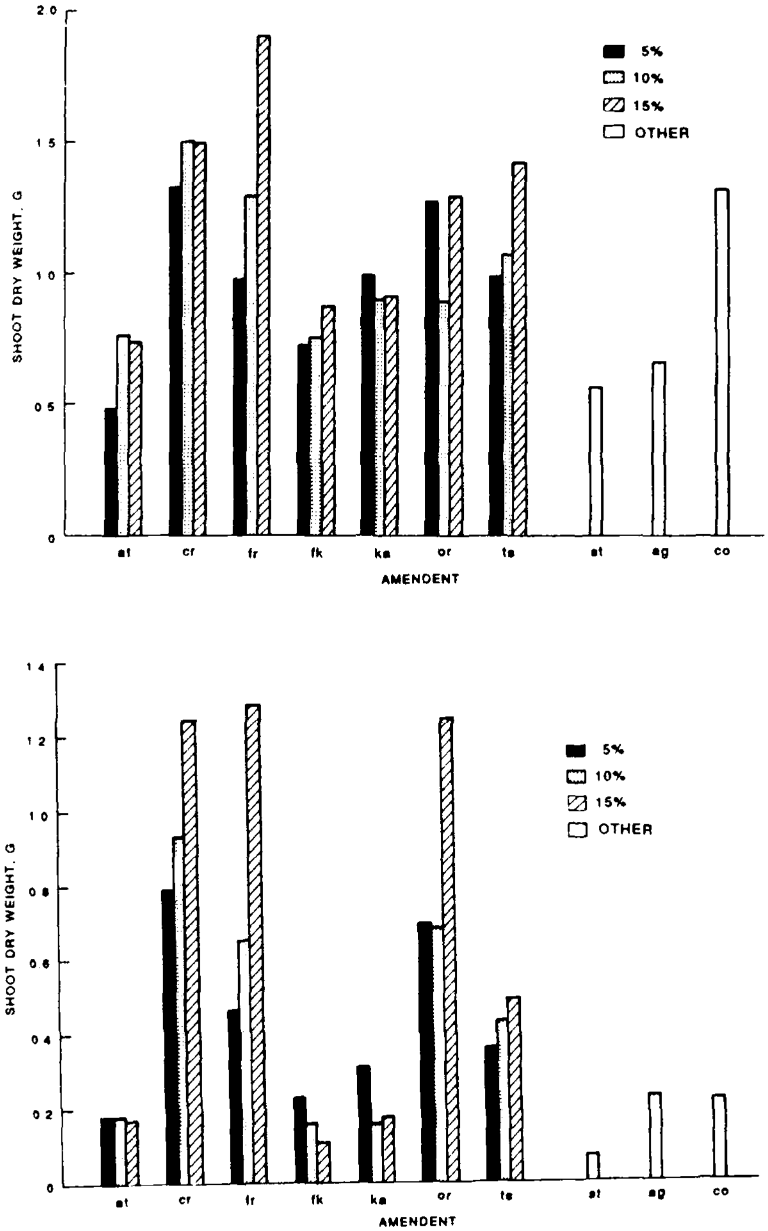


Figure 7. Yield of smooth brome grass grown (a) with, and (b) without VAM in sand plus the amendments Attagel 350 (AT), Creek-O-Nite (CR), Florco RVM (FR), Florkleen (FK), Kaolin RC-32 (KA), Oran-RVM (OR), topsoil (TS), Agrosoke (AG), and control (i.e. no amendment, CO).

Figure 8. 1988 field demonstration plot 148 days after planting. Small demonstration plot area is on the left, large demonstration plot area is on the right. Note plant growth (right background) within VAM/clay and VAM/clay/mulch amended areas.

Figure 9. Plant growth in the VAM/clay (foreground) and VAM/clay/mulch (background) amendment areas. Note greater amount of growth in VAM/clay/mulch area

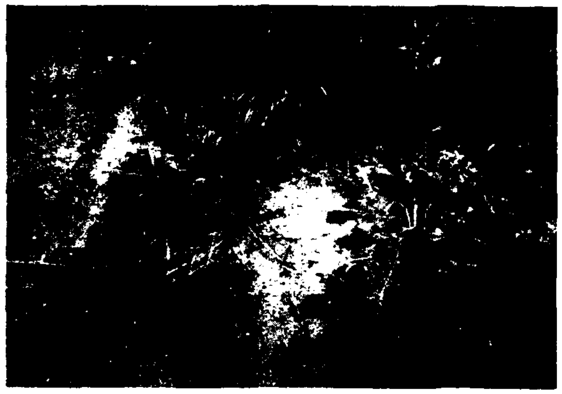


Figure 10. Example of plant growth in the VAM/clay amendment area

Figure 11. Example of plant growth in the VAM/clay/mulch amendment area

Figure 12. Plant growth in VAM/clay/mulch amendment area. The taller grass is giant foxtail